

in FIG. 4A. In FIG. 4B, the center of the low (triangles) and high (squares) frequency resonances as a function of bin number, each bin consisting of the average of 10 transients. From these data, the mean value of the central frequency for the two peaks was determined to be 140.566(4) and 281.082 (3) Hz, as indicated by the solid lines overlaying the data.

[0052] As mentioned above, homonuclear J-coupling between equivalent spins cannot be observed. In high-field NMR experiments, this is often overcome by differences in chemical shift between different functional groups. At low or zero magnetic field, where chemical shifts are unresolved or non-existent, homonuclear non-equivalence can occur through different heteronuclear J-coupling environments (see, e.g., ref 15). For example, in ethanol 1, $^{12}\text{CH}_3\text{-}^{13}\text{CH}_2\text{-OH}$, or ethanol 2, $^{13}\text{CH}_3\text{-}^{12}\text{CH}_2\text{-OH}$, the protons in the methyl and methylene groups couple to the ^{13}C nucleus differently, yielding observable effects due to homonuclear J-coupling. FIGS. 5A and 5B show experimental spectra for ethanol 2 and ethanol 1, obtained after averaging 475 and 210 transients, respectively. Simulated spectra, presented below the data, are in agreement with experiment. In the simulations, the values of coupling constants obtained from high-field measurements were used, which, for ethanol 1 are $J_{\text{HC}}^{(1)}=140.4$ Hz, $J_{\text{HC}}^{(2)}=4.6$ Hz and $J_{\text{HC}}^{(3)}=7.1$ Hz and for ethanol 2 are $J_{\text{HC}}^{(1)}=125.2$ Hz, $J_{\text{HC}}^{(2)}=2.4$ Hz and $J_{\text{HC}}^{(3)}=7.1$ Hz, where the superscript denotes the number of bonds separating the interacting nuclei. These spectra can be interpreted as follows: The Hamiltonian is dominated by the one-bond heteronuclear J-coupling. Hence, neglecting any other couplings, for ethanol 1, one expects a single peak at $3J_{\text{HC}}^{(1)}/2$ due to coupling between the ^{13}C nucleus and the triplet proton state of the methylene group. In ethanol 2, one expects two peaks at $J_{\text{HC}}^{(1)}$ and $2J_{\text{HC}}^{(1)}$ due to coupling between the ^{13}C nucleus and the doublet or quadruplet states of the protons on the methyl group. Homonuclear couplings and two-bond heteronuclear couplings result in a splitting of these peaks, as well as the appearance of a set of peaks at low frequencies.

[0053] As shown in FIG. 5, to the extent that the signal is above the noise level, experiment and simulation are in agreement. The positions of the multiplets are determined by the one-bond heteronuclear J-coupling and the splittings within the multiplets are due to homonuclear J-coupling and two-bond heteronuclear J-coupling.

[0054] Simulation and further experimental results not presented here indicate that spectra rapidly become quite complex in molecules such as doubly labeled ethanol, where there are multiple one-bond heteronuclear and homonuclear couplings. Future work is planned to explore methods for selective and broadband decoupling of heteronuclear scalar couplings for simplification of zero field spectra. Compounds containing nitrogen (present in many biologically relevant molecules) can be labeled with ^{15}N which will provide an additional means for manipulating the nuclear spin Hamiltonian. Lastly, while the present results were obtained in a zero field environment, spectral features in the complementary low field ($\sim 50\text{ }\mu\text{T}$) regime can also be observed with an atomic magnetometer. As demonstrated Savukov et al. in *J. Magn. Res.*, atomic magnetometers can also be used for direct detection of NMR in finite fields.

[0055] Finally, in the present work, the magnetometric sensitivity is about $200\text{ fT}/\sqrt{\text{Hz}}$, with a vapor cell volume of about 4.8 mm^3 . Laser intensity fluctuations are the dominant source of noise and are about a factor of 50 larger than photon shot noise. A straightforward path to improved sensitivity would

be to incorporate a second, low noise laser, and monitor optical rotation, which would cancel common mode noise. Fundamentally limiting the sensitivity of an atomic magnetometer is spin-projection noise (see, D. Budker, M. V. Romalis, Optical Magnetometry, *Nature Physics* 3 (2007) 227-234), and in Ledbetter et al., *Proc. Natl. Acad. Sci.*, it was estimated that, for millimeter-scale vapor cells with optimal values of parameters such as light power, cell temperature, and buffer gas pressure, spin-projection noise is on the order of $0.1\text{ fT}/\sqrt{\text{Hz}}$, indicating that there is still a great deal of room for improved magnetometric sensitivity. Hyperpolarization techniques such as dynamic nuclear polarization or parahydrogen-induced polarization can also be employed to yield much larger signals, making possible the detection of natural-abundance samples.

[0056] In conclusion, direct detection of pure J-coupling NMR at zero magnetic field using an optical atomic magnetometer has been demonstrated. For characteristic functional groups, such as $^{13}\text{CH}_3$, the zero-field spectrum is simpler than Earth-field spectra while retaining all information about the J-coupling network. Linewidths as low as 0.1 Hz were obtained, heteronuclear J-coupling constants with 4-mHz statistical uncertainty were measured, and homonuclear J-coupling was clearly observed. Zero-field relaxation rates can also easily be measured with only a single pulse. The sensitivity is sufficient to obtain simple spectra from $80\text{ }\mu\text{L}$ of fluid in a single shot. Further optimization of magnetometric sensitivity and geometry is expected to yield improved performance with detection volumes at the level of $1\text{ }\mu\text{L}$. It is anticipated that the technique described here will find wide use in analytical chemistry. Applications to multiplexed screening, assaying and identification of samples from chemistry to biomedicine with mobile, miniaturized devices are also envisaged. One particular application that is envisioned is in monitoring changes of scalar couplings in the products of enzyme catalyzed reactions.

Parahydrogen Enhanced Zero-Field Nuclear Magnetic Resonance

[0057] The present invention provides direct detection of zero-field NMR signals generated via parahydrogen induced polarization (PHIP), enabling high-resolution NMR without the use of any magnets. The sensitivity is sufficient to observe spectra exhibiting $^{13}\text{C}\text{-}^1\text{H}$ scalar nuclear spin-spin couplings (the so-called J-couplings) in compounds with ^{13}C in natural abundance in a single transient. The resulting spectra display distinct features that have straightforward interpretation and can be used for chemical fingerprinting.

[0058] The present invention removes the obstacles of low nuclear spin polarization and poor sensitivity of inductive pickup coils at low frequencies. The present invention can obtain high-resolution, high signal-to-noise ratio, zero-field NMR spectra that are rich in information. This facilitates the development of portable sensors for chemical analysis and imaging by elimination of cryogenically cooled superconducting magnets. Additionally, working in low or zero magnetic-field yields narrow lines and accurate determination of line positions, due to the high absolute field homogeneity and stability. These features have enabled chemical analysis via ^{129}Xe chemical shifts³ and spin-spin or J-couplings between $^1\text{H}\text{-}^{13}\text{C}$, $^1\text{H}\text{-}^{29}\text{Si}$, and $^1\text{H}\text{-}^{19}\text{F}$ in low- or zero-magnetic field^{4, 5, 6}. Atomic magnetometers^{7,8} and SQUIDS⁹ are sensitive to low-frequency signals, offering dramatically improved sig-